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Pharmacokinetics for Regulatory Risk Analysis: The Case of 1,1,1-Trichloroethane (Methyl Chloroform)

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A methodology for using physiologically based pharmacokinetic (PBPK) models to derive predicted safe concentrations of noncarcinogens in drinking water for humans based on experimentally determined no observed adverse effect levels (NOAELs) in animals is presented and applied to the case of 1,1,1-trichloroethane (methyl chloroform, MC). For each toxic endpoint and lowest corresponding NOAEL identified for MC, we considered a set of toxicologically plausible options regarding the presumed toxic agent and the metric for effective dose to target tissue. A four-compartment PBPK model for rodents was used to estimate corresponding effective doses to the animals used to obtain the experimental NOAELs. A five-compartment PBPK model was then applied, in conjunction with a multiroute (inhalation, ingestion, and dermal) human-exposure scenario, to calculate alternative concentrations of MC in drinking water predicted to result in corresponding effective doses to the same target tissues in humans. In the case of MC, the PBPK approach to interspecies and interroute extrapolation of toxicity data resulted in lower drinking water concentrations predicted to be nontoxic to humans than corresponding concentrations obtained using a traditional method for determining safe levels.

INTRODUCTION

The traditional approach to converting doses of a toxic substance administered to a laboratory animal to a dose in humans that elicits the same toxic response is based on the assumption that an applied dose relating to an effect or a no-observed-adverse-effect level (NOAEL) in animals may be used to predict a corresponding applied dose in humans. In this approach, one or more safety factors are applied to an experimentally determined NOAEL to account for uncertainties involved in interspecies and interroute extrapolations of observed toxicity (or nontoxicity).

As an alternative to this traditional approach, the use of physiologically based pharmacokinetic (PBPK) models that predict the uptake, metabolism, and excretion of volatile organic compounds (VOCs) in exposed animals or humans has been recommended as a better way to undertake interspecies and interroute extrapolations of tox-

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TABLE 1

METABOLISM OF MC IN RODENTS AFTER ORAL OR INTRAPERITONEAL (ip) EXPOSURE

Species (strain) Sex (No. of animals)	Applied dose (mg/kg)	Exposure protocol	Recovery period (hr)	Percentage of applied dose recovered in: Exhaled air MC	Exhaled air CO_2	Excreta (% U[a])	Carcass	Percentage of dose recovered as metabolites	Percentage of metabolites excreted in urine	Reference
Rat (F344); Male (4)	143	*Ad libitum* 8 hr (drinking water)	56	90.5	2.2	2.5 (2.2)	1.3	6.0	36.7	Reitz *et al.* (1985)
Rat (N/A[b]) Male, female (3)	69	Single ip	25	47.6	0.5	0.88 (0.85)	0.2	1.6	55.1	Hase *et al.* (1960)
Rat (OM[c]) Male (4)	3000	5 days/week, 4 weeks gavage[d]	48	85.1	0.9	2.1 (N/A)	1.2	4.2	—	Mitoma *et al.* (1985)
Mouse (B6C3F1) Male (4)	4000	5 days/week, 4 weeks (gavage[d])	48	92.9	2.0	3.4 (N/A)	0.7	6.1	—	Mitoma *et al.* (1985)

[a] (% U) = percentage of applied dose recovered as urinary metabolites.
[b] N/A = not available.
[c] OM = Osborne Mendel.
[d] Radiolabel administered as single gavage dose after treatment as specified.

icity for VOCs (NRC 1986, 1988). An analytic strategy for the application of PBPK models to regulatory risk analysis for VOCs suspected of being human carcinogens was previously described and applied to the case of trichloroethylene (TCE) (Bogen 1988). Here we outline a strategy for using PBPK analysis to derive safe drinking water concentrations for humans based on experimentally determined NOAELs in animals. The case of 1,1,1-trichloroethane (methyl chloroform, denoted MC) is used to illustrate the proposed PBPK approach. Data on the kinetics of MC metabolism and excretion are first reviewed. We then review the PBPK models that have been applied to study MC pharmacokinetics in rodents and humans. In particular, we summarize the four-compartment PBPK model of Ramsey and Andersen (1984), and a related five-compartment model, to facilitate our subsequent steady-state analysis of these PBPK models. Next, we describe the particular PBPK models we use subsequently for interspecies and interroute extrapolation of MC toxicity. We then review available data on MC toxicity and select three animal studies adequate to define alternative NOAELs for extrapolation to humans. Finally, we describe a "PBPK paradigm" for interspecies and interroute extrapolation of toxicity and apply this approach to derive a set of alternative safe concentrations of MC in drinking water based on data from the three selected studies of chronic MC toxicity in animals. The latter concentrations are compared with corresponding concentrations derived using a traditional approach to extrapolating classical toxicity from animals to humans.

MC METABOLISM AND EXCRETION KINETICS

MC absorbed into the body is eliminated primarily through the lungs unchanged. Metabolism of MC is limited; relatively small amounts are excreted in the urine as

TABLE 2

METABOLISM OF MC IN RODENTS AFTER INHALATION EXPOSURE

Species (strain); Sex (No. of animals)	Applied concentration (ppm)	Exposure protocol	Recovery period (hr)	Amount metabolized to CO_2 (mg/kg)	Amount excreted as urinary metabolites (mg/kg)	Total amount metabolized (mg/kg)	% of metabolites excreted in urine	Reference
Rat (Wistar); N/A[a] (20)	220	4 hr (1 day)	72	—	0.58	—	—	Eben and Kimmerle (1974)
Rat (Wistar); N/A (20)	440	4 hr (1 day)	72	—	0.97	—	—	Eben and Kimmerle (1974)
Rat (Wistar); Male, female (6)	200	8 hr (1 day)	48	—	3.6	—	—	Ikeda and Ohtsuji (1972)
Rat (F344); Male (4)	150	6 hr (1 day)	72	0.29	0.69	1.2	57.5	Schumann *et al.* (1982a)
	1500	6 hr (1 day)	72	1.1	1.4	2.4	41.2	
Rat (F344); Male (4)	1500	6 hr (1 day)	72	1.5	3.4	5.3	64.2	Schumann *et al.* (1982b)
	1500	6 hr, 5 days/week, 16 months	72	2.3	3.5	6.2	56.6	
Mouse (B6C3F1); Male (4)	150	6 hr (1 day)	72	0.29	2.0	3.1	66.2	Schumann *et al.* (1982a)
	1500	6 hr (1 day)	72	0.86	3.9	5.7	68.9	
Mouse (B6C3F1); Male (4)	1500	6 hr (1 day)	72	12.8	11.6	28.3	41.0	Schumann *et al.* (1982b)
	1500	6 hr/day, 5 days/week, 16 months	72	14.6	13.6	32.3	42.1	

[a] N/A = not available or reported.

trichloroacetic acid (TCA), trichloroethanol (TCEL), and trichloroethanol-glucuronide (TCEL-G). In addition, some MC is transformed to carbon dioxide (CO_2) and eliminated in exhaled air. Mass-balance studies using ^{14}C-MC have shown that pulmonary elimination of unchanged parent compound is the most significant route of MC excretion (see Table 1). Although inhalation experiments have not specified the total applied dose, the pattern of metabolite recovery parallels that observed after oral dosing (Table 2). Data from Tables 1 and 2 indicate that mice excrete between 41 and 68.9% and rats excrete between 36.7 and 64.2% of an applied MC dose as urinary metabolites. Therefore, a considerable fraction of total MC metabolites are eliminated by nonurinary routes in these rodents. Corresponding values of the proportion of MC metabolites excreted in urine are not available for humans. However, this proportion may be estimated by considering the corresponding reported percentages for rats. Thus, for the purpose of calculating the effective dose to humans (see below), we make the assumption that approximately 50% of all MC metabolites are excreted in urine by humans. This value is similar to the midpoint of the corresponding ranges observed for perchloroethylene and slightly less than that reported for trichloroethylene (Bogen and McKone, 1988; Bogen 1988). Urinary metabolites of MC collected after voluntary or occupational exposure to MC demonstrate that humans, like ro-

TABLE 3

METABOLISM OF MC IN HUMANS

Exposure concentration (ppmv)	No. of individuals	Exposure duration (hr)	Recovery period	Total amount of MC recovered as TCEL (mg)	Total amount of MC recovered as TCA (mg)	Estimated total uptake (mg)	Estimated % of uptake recovered as urinary metabolites[a]	Reference
4	10	Chronic occupational	"Latter half" of work week	1.8	0.8	—	—	Seki *et al.* (1975)
25	26	"	"	2.2	3.4	—	—	Seki *et al.* (1975)
53	10	"	"	13.9	5.0	—	—	Seki *et al.* (1975)
72	3	8	12 days	18.2	5.2	281.3[b]	7.3	Humbert and Fernandez (1977)
213	2	8	12 days	50.7	15.0	916.5[b]	4.8	Humbert and Fernandez (1977)
70 (at rest)	6	4	70 hr	6.0	1.7	192.5[c]	4.0	Monster *et al.* (1979)
142 (work load)	6	4	70 hr	11.5	3.0	537.5[c]	2.7	Monster *et al.* (1979)
345 (at rest)	6	4	70 hr	11.5	3.0	429.2	3.4	Monster *et al.* (1979)
35	6	6	9 days	4.9	3.2	101.6[d]	5.0 (to 6.0)	Nolan *et al.* (1984)
350	6	6	9 days	12.7	24.4	1005.0	5.0 (to 6.0)	Nolan *et al.* (1984)

[a] Calculated as total urinary metabolites (mg)/total uptake MC (mg) × 100.

[b] Estimated by Humbert and Fernandez (1977) by the following formula: uptake = [(8 hr) $Conc_{inspired}$ − $\sum_0^8 Conc_{alveolar}(t)dt$] (alveolar ventilation rate).

[c] Estimated by Monster *et al.* (1979) from the following formula: uptake = ($Conc_{inspired}$ − $Conc_{exhaled}$) (breathing rate) (observation time).

[d] Values were estimated from a physiologic model (see text), but were based on actual recovery of TCA and TCEL.

dents, metabolize only a small fraction of large applied MC doses and that urinary metabolites comprise an important component of MC metabolites in humans (see Table 3).

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS APPLIED TO MC

To simulate the uptake, metabolism, and excretion of MC in humans, Caperos *et al.* (1982) adapted a PBPK model, developed by Fernandez *et al.* (1977), which consisted of a pulmonary compartment, an organ responsible for metabolism (the liver), and three additional compartments: vessel-rich (richly perfused) tissue, muscle and skin (poorly perfused) tissues, and fat (very poorly perfused) tissues. In the modified version of this model used by Caperos *et al.* (1982), the liver was not treated as a separate compartment. Instead, they assumed that a specific amount of blood (0.025 liter) was completely cleared of MC every minute. This value was calculated from the experimental data of Humbert and Fernandez (1977), who measured concentrations of MC in blood and exhaled air and the production of urinary metabolites in five male volunteers exposed to 72 or 213 ppmv MC for 8 hr. Their model accounted for the mass-balance relationships of MC in the compartments. MC leaving the body through the lungs, and MC eliminated as metabolites in the urine. The uptake and

excretion kinetics were assumed to be linear and governed by tissue volumes, tissue-specific blood volumes, and tissue-specific blood perfusion and tissue-gas partition coefficients. The values for kinetic parameters governing excretion of the MC metabolites TCEL, TCEL-G, and TCA used by Caperos *et al.* (1982) were the same as those used by Fernandez *et al.* (1977) to describe the excretion of the same metabolites of TCE; the latter metabolic rate constants were estimated from human pharmacokinetic data.

When the PBPK model of Caperos *et al.* (1982) was used to simulate the uptake and elimination of MC with an exposure scenario that matched the experimental conditions used by Humbert and Fernandez (1977), the model was able to fit the experimental data reasonably well.

Nolan *et al.* (1984) studied volunteers who were exposed to MC (six male Caucasians weighing about 85 kg inhaled 35 or 350 ppmv of MC for 6 hr, and blood and urine samples were collected for 9 days postexposure and analyzed for MC, TCEL, and TCA). They then compared their experimental results with the disposition of MC predicted by a PBPK model similar to that used by Caperos *et al.* (1982), except that the Nolan *et al.* model assumed that all metabolism occurred in well-perfused tissue. This pharmacokinetic model predicted MC concentrations in expired air nearly identical to those measured from the volunteers over the 9-day postexposure period. The model also accurately estimated blood concentration of MC and urinary excretion of TCEL and TCA observed over the same period.

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The Ramsey–Andersen PBPK Model

Recently, the National Research Council (NRC) considered the use of PBPK models to facilitate dose–route extrapolation when using inhalation toxicity data to set safe drinking water limits (NRC, 1986). A range of issues was considered in this study, which included illustrative examples using the PBPK approach for dose–route extrapolation from rats to humans for noncarcinogenic toxicity associated with exposure to TCE and benzene. The pharmacokinetic model used was developed by Ramsey and Andersen (1984) to describe the uptake, metabolism, and excretion of styrene in rats and humans. The structure of the model is shown in Fig. 1, and its parameter definitions are given in Table 4. This type of model has been applied to the study and prediction of animal and human pharmacokinetics for other VOCs, including benzene, methylene chloride, tetrachloroethylene, and TCE (NRC, 1986; Gargas *et al.*, 1986; Andersen *et al.*, 1987; U.S. EPA, 1986a; Reitz and Nolan, 1986; Hattis *et al.*, 1987; Ward *et al.*, 1988; Bogen and McKone, 1988; Bogen, 1988), as well as MC (Reitz *et al.*, 1987). The model consists of a set of differential equations that describe the rate of change of the amount of absorbed chemical present in each of four physiologically realistic tissue compartments, which are assumed to be ideally well-mixed at any given time. Metabolism is presumed to occur solely in the liver through a saturable enzymatic process with Michaelis–Menten kinetics.

According to the Ramsey–Andersen model, pulmonary uptake of a chemical occurs continuously such that alveolar concentration, C_a, is in instantaneous equilibrium with arterial blood governed by the blood/air partition coefficient, P_b, in accordance with the relation $C_a = B_a/P_b$. Similarly, the concentrations, C_i, of chemical in

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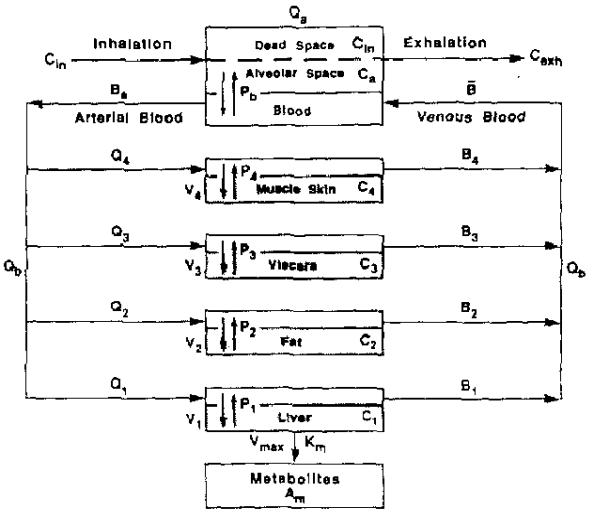

FIG. 1. Schematic diagram of physiologically based pharmacokinetic (PBPK) model for inhalation of volatile organic compounds. The model assumes that four "well-stirred" compartments or tissue groups collect inhaled compound at rates governed by air concentration (C_{in}), air and blood flows (Q), blood concentrations (B), compartment volumes (V), tissue/blood partition coefficients (P), and metabolic parameters (V_{max}, K_m).

each tissue compartment are presumed to be in instantaneous equilibrium with the concentrations, B_i, in venous blood exiting the corresponding tissue, governed by the corresponding tissue/blood partition coefficients such that $B_i = C_i/P_i$. The amount of chemical in any given tissue compartment is given by $A_i = C_i V_i$. For notational convenience, the dependence of state variables (C's, B's, and A's) on time t is suppressed.

The model is based on the assumption that chemical delivered to the lung via respiratory retention and returning venous blood is balanced by the chemical mass exiting the lung via exhalation and arterial blood, which implies that

$$Q_a(C_{in} - C_a) = Q_b(B_a - \bar{B}), \tag{1}$$

and

$$B_a = \frac{Q_a C_{in} + Q_b \bar{B}}{(Q_a/P_b) + Q_b}. \tag{2}$$

Similarly, the concentration $\bar{B}$ in venous blood returning from each compartment is presumed to be the instantaneous flow-weighted average:

$$\bar{B} = \frac{1}{Q_b} \sum_{i=1}^{4} Q_i B_i. \tag{3}$$

For the nonmetabolizing tissues, the amount of chemical entering the ith compart-

TABLE 4

COMPARTMENT AND PARAMETER DEFINITIONS FOR THE RAMSEY–ANDERSEN PBPK MODEL AND A RELATED MODEL (SEE TEXT)

Abbreviations	Definition	Units
C_{in}	Concentration in air inhaled	mg/liter air
C_a	Concentration in alveolar air	mg/liter air
C_{exh}	Measured concentration in expired breath	mg/liter air
Q_a	Alveolar ventilation rate	liter air/hr
Q_b	Cardiac output ($=\Sigma Q_i$)	liter blood/hr
P_b	Blood/air partition coefficient	liter air/liter blood
B_a	Arterial blood concentration	mg/liter blood
$\bar{B}$	Venous blood concentration	mg/liter blood
A_m	Amount metabolized in liver	mg
Q_i	Blood flow rate to compartment i	liter blood/hr
V_i	Volume of compartment i	liter (=kg)
C_i	Concentration in compartment i	mg/liter
B_i	Concentration in venous blood leaving compartment i	mg/liter blood
A_i	Amount in compartment i	mg
P_i	Tissue/blood partition coefficient for compartment i	liter blood/liter tissue i
V_{max}	Maximum metabolic rate	mg/hr
K_m	Apparent Michaelis constant $[B_1(dA_m/dt \quad V_{max}/2)]$	mg/liter blood
R_1	Rate of ingestive absorption	mg/hr
R_2	Rate of dermal absorption	mg/hr

Compartmental subscripts:

$i = 1$	Liver (metabolizing tissue group)
2	Fat tissue (very poorly perfused)
3	Richly perfused tissues (brain, kidney, viscera)
4	Poorly perfused tissues (muscle, skin)
5	Skin (if compartment 4 represents muscle only)

ment via arterial blood is set equal to the amount gained by that compartment plus the amount leaving in venous blood; i.e., it is assumed that

$$\dot{B}_i = \frac{Q_i}{V_i P_i}(B_a - B_i) \qquad i = 2, 3, 4, \tag{4}$$

where dot notation is used here, and below, to represent differentiation with respect to time (i.e., $\dot{B}_i = dB_i/dt$, etc.). The rate at which chemical is metabolized in liver is given by the Michaelis–Menten relation

$$\dot{A}_m = \frac{V_{max} B_1}{K_m + B_1}, \tag{5}$$

in which K_m is the concentration in venous liver blood at which $\dot{A}_m$ equals half its maximum value, V_{max}. Thus, venous liver blood concentration is described by the relation

$$\dot{B}_1 = \frac{Q_1}{V_1 P_1}(B_a - B_1) - \frac{\dot{A}_m}{V_1 P_1}. \tag{6}$$

The system of Eqs. (2)–(6) represents the PBPK model for inhalation of a volatile

chemical, and for any given time its compartmental quantities A_i or corresponding concentrations C_i or B_i are found by simultaneous numerical integration of the system.

Exposure by routes other than inhalation is easily incorporated into this model. Because blood draining the stomach, small intestine, and colon passes through the hepatic portal vein, exposure to VOCs via ingestion is modeled simply by assuming a direct introduction of the ingested mass into the liver compartment (NRC, 1986). The latter introduction can be assumed to reflect a first-order infusion process, the approach taken in NRC (1986), or it may be modeled more simply as a constant infusion for a specified period of time into the liver at a rate R_1 (mg/hr) by adding the constant $R_1/(V_1P_1)$ to the right side of Eq. (6). Similarly, dermal absorption may be modeled by splitting compartment 4 (muscle plus skin) in the model described above into separate compartments for muscle (new compartment 4) and skin (new compartment 5), and by letting $i = 1, \ldots, 5$ in Eq. (3). A constant absorption at a rate R_5 (mg/hr) into the skin compartment over a specified period would then be modeled by adding the term $R_5/(V_5P_5)$ to the right side of Eq. (4) with $i = 5$.

Analysis of PBPK System at Steady State

In the context of environmental regulation, very low-level, continuous exposure scenarios are typically of concern. The following is an analysis of how the PBPK model just described behaves under steady-state, respiratory exposure conditions. Steady-state ingestive and dermal exposure, using the constant infusion assumptions described earlier, are also considered for the purpose of comparison and because the mixed exposure case represents a scenario of human environmental exposure that is presumed to be the case in our dose-response assessment.

Because at steady state $B_i = B_a$ for $i = 2, 3, 4$, and $B_5 = B_a + R_5/Q_5$, Eq. (3) reduces under steady-state conditions to

$$\bar{B} = \frac{1}{Q_b}[Q_1B_1 + R_5 + B_a(Q_b - Q_1)], \tag{7}$$

so that Eq. (2) reduces to

$$B_a = \frac{Q_aC_{in} + Q_1B_1 + R_5}{(Q_a/P_b) + Q_1}. \tag{8}$$

Also at steady state, Eq. (6), modified as described above to reflect constant ingestive infusion, reduces to the form

$$\frac{V_{max}B_1}{K_m + B_1} = Q_1(B_a - B_1) + R_1, \tag{9}$$

so that the solution for venous liver blood concentration, given input C_{in}, is the quadratic root:

$$B_1 = Y + \sqrt{Y^2 + Z}, \tag{10a}$$

in which

$$Y = \frac{1}{2}[P_b(C_{in} + R_s/Q_a) + (R_t - V_{max})X - K_m], \quad (10b)$$

$$X = \left(\frac{P_b}{Q_a} + \frac{1}{Q_t}\right), \quad (10c)$$

and

$$Z = K_m(P_b(C_{in} + R_s/Q_a) + R_tX). \quad (10d)$$

The steady-state metabolic rate is thus given by using Eqs. (10a)–(10d) to evaluate B_1 in Eq. (5).

PBPK MODELS USED TO EXTRAPOLATE MC TOXICITY BETWEEN SPECIES

Recently, Reitz *et al.* (1987) used the PBPK model of Ramsey and Andersen (1984) to study the disposition of MC in rats, mice, and humans, using the corresponding model-parameter values listed in Table 5. This model was used to predict the kinetic profiles of MC in blood and expired air in each species as well as the total amount of MC metabolized. Model predictions were compared with the data of Schumann *et al.* (1982a) by calculating the ratio of model-generated values to actual experimental values. In young rats, the model was remarkably successful in predicting the amount metabolized (μmol) after exposure to 150 or 1500 ppmv MC (ratio of 1.0). The ratio between model predictions and actual data for body burden (0.76 to 0.91) and for the concentration in liver (0.73 to 1.0) also showed close agreement. The analogous ratios for mice ranged from 0.95 to 1.03 (amount metabolized), 0.63 to 0.94 (body burden), and 0.68 to 0.83 (concentration in liver). The time course of elimination of MC from venous blood was also well described by the PBPK model for both species.

A similar predictive ability was demonstrated when Reitz *et al.* (1987) used their PBPK model, parameterized for humans, to estimate the degree of MC metabolism expected after simulated MC exposures identical to those actually administered to humans in the Nolan *et al.* (1984) study discussed above (2.47 and 18.6 mg eq MC as metabolites were predicted for the low- and high-exposure groups, respectively, whereas Nolan *et al.* measured 4.12 and 31.3 mg eq MC as urinary metabolites).

Later we shall apply PBPK models, like the model parameterized by Reitz *et al.* (1987) just described, to rodent- and human-exposure scenarios relevant to dose-response assessment for MC. We describe these models here to highlight their relationship to the model validated by Reitz *et al.* (1987). Physiologic and metabolic parameter values that we use for rats and mice are taken directly from those given by Reitz *et al.* (1987) and listed in Table 5. Because there are no experimental estimates of blood/air or tissue/blood partition coefficients for MC in gerbils (necessary for our dose-response assessment), our PBPK analysis for gerbils uses the mouse partition coefficients listed in Table 5. The parameter values that Reitz *et al.* used for mice (respiration rate, blood flow, and metabolic clearance rate) were obtained by scaling

TABLE 5

PARAMETER VALUES USED IN PBPK MODEL FOR MC

Parameter	Unit	Mouse[a]	Rat[a]	Gerbil[b]	Experimental human male[c]	Reference human male[c]
W (body weight)	kg	0.029	0.215	0.059	83	70.0
Q_a	liter/hr	1.26	5.11	2.07	330.7	378.0
Q_b	liter/hr	1.26	5.11	2.07	330.7	372.0
P_b		10.8	5.76	10.8	2.53	2.53
Q_i/Q_b i = 1		0.24	0.24	0.24	0.24	0.26
2		0.02	0.05	0.02	0.09	0.05
3		0.53	0.53	0.53	0.53	0.44
4		0.21	0.18	0.21	0.14	0.235
5		—	—	—	—	0.015
V_i/W i = 1		0.04	0.04	0.04	0.026	0.026
2		0.04	0.07	0.04	0.195	0.19
3		0.05	0.05	0.05	0.064	0.05
4		0.79	0.76	0.79	0.635	0.583
5		—	—	—	—	0.037
P_i i = 1		0.796	1.49	0.796	3.40	3.40
2		24.4	45.7	24.4	104.0	104.0
3		0.796	1.49	0.796	3.40	3.40
4		0.292	0.547	0.292	1.25	1.25
5		—	—	—	—	53.0
V_{max}	mg/hr	0.0222	0.0904	0.0356	5.84	20.7
K_m	mg/liter	6.43	6.43	6.43	6.43	6.43

[a] Values taken from Reitz *et al.* (1987). Metabolic parameters for rats obtained from data of Schumann *et al.* (1982a), and metabolic parameters for mice and humans were calculated by scaling the ratio of these body weights to rat body weight to the 0.7 power. Physiologic parameters cited by Reitz *et al.* (1987) are from Caster *et al.* (1956), Davis and Mapleson (1981), and ICRP (1975).

[b] Gerbil body weight obtained by averaging values cited by Rosengren *et al.* (1985). V_{max}, Q_a, and Q_b were calculated by scaling data from Schumann *et al.* (1982a), as noted in (a).

[c] Reference physiologic parameters (except Q_a, Q_b, and V_b) for adult males from U.S. EPA (1988b). Partition coefficients (except P_b) and K_m from Reitz *et al.* (1987). See text for derivation of V_{max}, Q_a, Q_b, and V_b.

the values from rats to the 0.7 power of the ratio of mouse-to-rat body weight. The same approach was used to obtain corresponding values for gerbils shown in Table 5.

Table 5 also lists parameters of a five-compartment PBPK model, adapted from the Ramsey–Andersen model, which is incorporated into our interspecies and exposure-route extrapolations of MC pharmacokinetics and toxicity. To model dermal uptake of MC, a skin compartment was included in the Ramsey–Andersen PBPK model, as described earlier. For this purpose, it was assumed that the skin comprises 2.6 kg of a 70-kg reference male human (ICRP, 1975), or 6% of the reference value for the undifferentiated compartment representing poorly perfused tissues. Blood perfusion to the skin was adjusted proportionally. Other parameter values used in our PBPK model for a reference 70-kg adult are given in Table 5. Except for the skin compartment, the tissue-specific volumes and blood-perfusion rates as well as the cardiac output were taken from U.S. EPA (1988a). The alveolar ventilation rate of 378 liters/

hr (6.3 liters/min) represents the average value obtained from five participants in a recent study (Salzano *et al.*, 1984) reviewed by the U.S. EPA (1988a). The human blood/air and tissue/blood partition coefficients for MC as well as the value of K_m are those given by Reitz *et al.* (1987).

The value of V_{max} for a reference 70-kg human shown in Table 5 is approximately four times greater than the V_{max} used by Reitz *et al.* (1987). This adjustment was made, first, to account for the fact that their model with their V_{max} (which was scaled from the corresponding empirically fit value for rats) predicted a total metabolized dose observed by Nolan *et al.* (1984) in experimental human subjects that was too low by a factor of about 2. The Reitz *et al.* (1987) model also (and to a slightly greater degree) underestimated the extent of urinary metabolite production measured by Humbert and Fernandez (1977) in experimental human subjects. Second, data from animal studies clearly demonstrate that urinary metabolites account for only 36.7 to 68.9% of total metabolism after exposure to MC (see Tables 1 and 2). For example, significant amounts of ^{14}C have been recovered in the feces and carcass or in expired air as CO_2 after treatment with radiolabeled MC. We used the midpoint of the experimental range cited above to estimate that urinary metabolites account for about 50% of total metabolites. This estimate implies that the Reitz *et al.* value for V_{max} in humans should be doubled to account for this relationship. Considering both of these adjustments to V_{max} and scaling for application to a 70-kg adult, we corrected the Reitz *et al.* (1987) value by multiplying it by a factor of $4(70/83)^{0.7} = 3.55$.

SELECTION OF DATA ON MC TOXICITY FOR DOSE-RESPONSE ASSESSMENT

A NOAEL is the highest concentration level or dose that has not induced a statistically significant adverse effect in the most susceptible species of animal tested. Data that define a NOAEL are typically selected from those studies that have examined the most sensitive indicator of toxicity, used sufficient numbers of animals, and have documented evidence of a dose-response relationship (U.S. EPA, 1980, 1985a,b, 1986b, 1988b). In the absence of adequate human-toxicity data, information from animal studies can be used to estimate an acceptable daily intake (ADI) and corresponding drinking water concentration that should not cause adverse health effects in humans by application of an appropriate safety factor either directly to the NOAELs identified (U.S. EPA, 1980, 1985a,b, 1986b, 1988b; Dourson and Stara, 1983) or to corresponding effective doses identified through PBPK analysis.

None of the available studies of MC toxicity in humans used sufficiently sensitive measurements of toxicity to characterize clearly a human NOAEL for MC (Seki *et al.*, 1975; Stewart *et al.*, 1969, 1975; Maroni *et al.*, 1977; Kramer *et al.*, 1978). Data from studies on MC toxicity in animals that define adequately documented NOAELs are summarized in Table 6. Based on the criteria specified above, we determined that the lowest adequately documented NOAELs for MC are defined by data from the studies by NTP (1987b), Rosengren *et al.* (1985), and McNutt *et al.* (1975), which are reviewed briefly below.

NTP (1987b). Two studies of reproductive toxicity in animals found evidence of adverse effects when relatively low concentrations of MC were administered in drink-

Species (strain
n[a]

Rat (SD[b])
n = 5 to 6

Rat (CD[c])
n = 18 to 22

Rat (F
n = 20

Gerbil
n = 24

Mouse
(B6C3F1)
n = 160
Rat (SD[b])
n = 160

Rat (SD[b])
n = 96

Rat (SD[b])
n = 10

Mouse (CF-
n = 300

[a] n = nu
[b] SD = S
[c] CD = C
[d] W = W

TABLE 6

ANIMAL-TOXICITY STUDIES THAT DEFINE AN APPARENT NOAEL FOR MC

Species (strain) n^a	Administered concentration or dose	Route of administration	Exposure protocol	Effect	Reference
Rat (SD[b]) n = 5 to 6	1000 ppm	Drinking water	*Ad libitum* 30 days pretreatment 21 days gestation 21 to 23 days lactation	30% incidence, cardiac defects in pups	Dapson *et al.* (1984)
Rat (CD[c]) n = 18 to 22	3, 10, 30 ppm	Drinking water	*Ad libitum* 27 days pretreatment 21 days gestation 21 days lactation	Increased fetal mortality, implantation through pnd 1 (30 ppm, $P \leq 0.01$)	NTP (1987b)
Rat (H[d]) n = 20	204 ppmv	Inhalation	8 hr/day, 5 days/week, 14 weeks	No apparent adverse effect	Eben and Kimmerle (1974)
Gerbil n = 24	70, 210, 1000 ppmv	Inhalation	24 hr/day, 3 months; 4 months exposure-free	Increased GFA protein, cerebral cortex (210, 1000 ppmv; $P \leq 0.05$, $P \leq 0.01$)	Rosengren *et al.* (1985)
Mouse (B6C3F1) n = 160	150, 500, 1500 ppmv	Inhalation	6 hr/day, 5 days/week 24 months	No apparent adverse effect	Quast *et al.* (1984)
Rat (SD[b]) n = 160	150, 500, 1500 ppmv	Inhalation	6 hr/day, 5 days/week, 24 months	Significantly decreased body weight (females, 1500 ppmv) and increased hepatic congestion (males, 1500 ppmv)	Quast *et al.* (1978)
Rat (SD[b]) n = 96	875, 1750 ppmv	Inhalation	6 hr/day, 5 days/week, 12 months	Significantly increased incidence of hepatocellular degeneration and/or necrosis (875 ppmv), dilated ventricles of heart (875 ppmv), minimal chronic nephropathy (1750 ppmv)	Quast *et al.* (1978)
Rat (SD[b]) n = 10	500 ppmv	Inhalation	6 hr/day, 4 days	Decrease in brain RNA and protein content	Savolainen *et al.* (1977)
Mouse (CF-1) n = 300	250, 1000 ppmv	Inhalation	24 hr/day, 14 weeks	Increased liver weight and liver triglyceride content (1000 ppmv, $P \leq 0.01$); changes in cytoplasm of hepatocytes (250 and 1000 ppmv)	McNutt *et al.* (1975)

[a] n = number of animals per dose or concentration.
[b] SD = Sprague–Dawley.
[c] CD = Charles River, Sprague–Dawley.
[d] W = Wistar.

ing water. In an abstract, Dapson *et al.* (1984) reported that the offspring of rats given MC in drinking water had a substantially greater incidence of cardiac defects than the offspring of controls. Few details of the exposure protocol were provided in the abstract, and the National Toxicology Program (NTP 1987a,b) subsequently sponsored two in-depth studies of the teratogenic and postnatal toxicity of MC administered in drinking water. Treatment of rats with 3, 10, or 30 ppm MC in drinking water (*ad libitum* throughout gestation) produced no evidence of teratologic effects (NTP, 1987a). A companion study (NTP, 1987b) of similarly exposed rats was designed to evaluate the postnatal effects of MC (see Table 6). In this study, there were no treatment-related effects on fertility, length of gestation, or a number of other indices of toxicity. However, among litters of MC-treated dams from the highest dose group, compared to control pups, there was a significant increase in percentage mortality from implantation to postnatal day (pnd) 1.[1]

A conservative interpretation of the latter NTP (1987b) finding just summarized is that it defines a NOAEL of 10 ppm MC in drinking water, e.g., according to U.S. EPA (1986b) criteria. However, the biological significance of, and hence the overall weight accorded to these data in the context of regulatory toxicology, must be evaluated critically in light of the potentially contradictory results of the similar NTP (1987a) study.

Rosengren et al. (1985). MC and other structurally related chlorinated hydrocarbons have been used as surgical anesthetics, and exposure to large concentrations can cause CNS depression. CNS effects have typically been measured by gross indices of toxicity such as ataxia, lethargy, stupor, and loss of consciousness. It has been widely held that these effects were completely reversible and posed no long-term threat to the health of animals or humans (Stewart, 1968; U.S. EPA 1984, 1985a). Rosengren *et al.* tested whether or not MC might cause long-lasting or permanent adverse effects on the CNS using gerbils exposed continuously to MC by inhalation over a period chosen to allow sufficient time for neuronal recovery after transient damage (see Table 6). Based on evidence from human and animal studies, Rosengren *et al.* examined the effects of MC on the astroglial cell population of the brain. Previous reports had established that a broad variety of viral, bacterial, physical, and chemical agents that damage the brain induce formation of fibrils in the astroglial cells (astrocytes) of damaged tissue (i.e., damage to astrocytes is followed by the formation of astroglial fibers). Astroglial fibrils are characterized by the presence of a unique substance, glial fibrillary acidic protein (GFA). GFA is the main protein subunit of fibrillary astrocytes, and it has been used as a biochemical marker for the detection of astroglial fibrils. Another astroglial protein, S-100, has also been used to track chemically induced changes in the astroglia.

[1] In addition, 10 of 28 pups found dead from MC-treated mothers (all three dose groups) had patent ductus arteriosus, which was not observed in control pups. While closure of the ductus arteriosus normally occurs within a few hours of birth, this closure is reversible in rats up to 5 days after birth. Because the pups in question were 1 day of age (when closure of the ductus arteriosus is still reversible), the NTP (1987b) did not consider the association between incidence of patent ductus arteriosus and increased early mortality to be biologically significant. It should be noted that the patent ductus arteriosus and other cardiac malformations reported by Dapson *et al.* (1984) were observed in 21-day-old pups—well after the time period in which normal, reversible reopening of the ductus arteriosus can occur.

Rosengren *et al.* found that gerbils exposed to 210 or 1000 ppmv MC had significantly greater concentrations of GFA in the sensorimotor cerebral cortex than untreated controls. Brain weights of the high-dose animals (1000 ppmv) were found to be significantly less than those of controls, and gerbils in the 210 ppmv group were found to have significantly less S-100 protein in the frontal cerebral cortex and less total protein in the occipital cerebral cortex. Rosengren *et al.* concluded that the significantly increased concentrations of GFA in the gerbil brain after exposure to 210 or 1000 ppmv MC are compatible with astrogliosis in this region. Because this response to MC was not observed in animals exposed to 70 ppmv, these data indicate a NOAEL of 70 ppmv.

McNutt et al. (1975). Previous analyses of animal dose-response toxicity data on MC have cited the data of McNutt *et al.* (1975) for definition of a subchronic lowest observed adverse effect level (LOAEL) or NOAEL, depending on the interpretation of results (U.S. EPA 1984, 1985a). This large-scale hepatotoxicity study involved mice exposed to MC continuously by inhalation (see Table 6). Animals exposed to 1000 ppmv had a significant increase in liver weight compared to controls at five sampling periods during exposure; liver weight per 100 g body wt and liver triglyceride content were also significantly greater in this dose group. These effects were not seen in low-dose mice (250 ppmv). Exposure to MC caused various cytoplasmic alterations in hepatocytes, including vesiculation of the rough endoplasmic reticulum, an increase in the smooth endoplasmic reticulum, and an increase in the number of microbodies (peroxisomes). In the group exposed to 250 ppmv MC, these cytoplasmic changes were classified as "mild to minimal," while exposure to 1000 ppmv MC resulted in "severe" changes. The U.S. EPA (1985a) used these data to define a subchronic inhalation NOAEL of 250 ppmv for mice exposed to MC.

PBPK APPROACH TO EXTRAPOLATING TOXICITY BETWEEN SPECIES AND EXPOSURE ROUTES

The PBPK paradigm for deriving an "expected no-adverse-effect level" (ENAEL) or ADI for humans based on experimental NOAELs in animals involves the assumptions and analytic steps depicted in Figs. 2 and 3. Figure 3 is meant to be a generic elaboration of the scheme illustrated in Fig. 2. The starting point required for this procedure is the set of three experimentally determined NOAELs that were selected from a series of potentially relevant studies reviewed in the preceding discussion. Each of these NOAELs pertains to a given animal species, a single applied dose or concentration of MC, and one specific endpoint (hepatic, CNS, or reproductive toxicity).

For each endpoint, there are several sets of toxicologically plausible inference options regarding the presumed toxic agent and appropriate metric for effective dose to relevant target tissue. In the context of this paper, the agent is the chemical species or process that is presumed to be the proximate cause of the observed toxicity, while the metric reflects how the agent causes damage in a given target tissue. Figure 3 lists two different metrics that are considered in this analysis, both of which are inherently flawed. Use of the time-weighted average (TWA) concentration in target tissue assumes that the agent in question does not have a threshold of action and that all of a

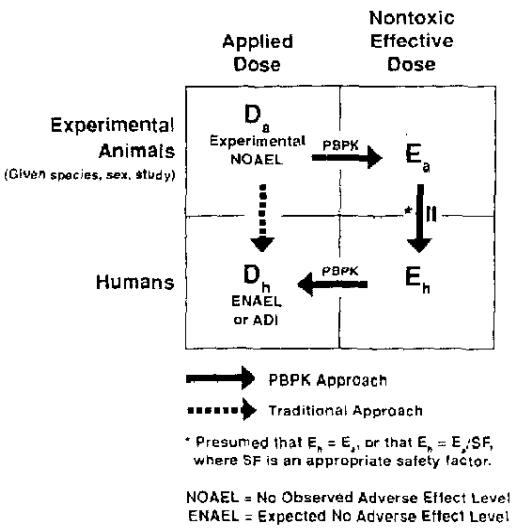

FIG. 2. PBPK paradigm for predicting nontoxic doses in humans based on extrapolation from NOAELs in experimental animals.

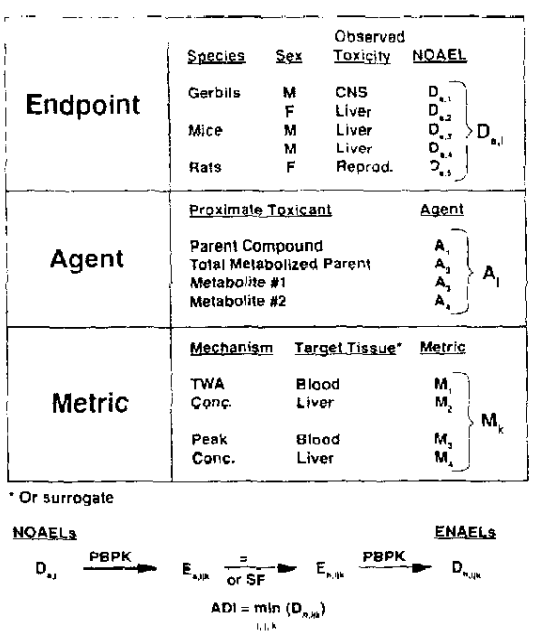

FIG. 3. Elements involved in the application of the PBPK paradigm for interspecies dose-response extrapolation illustrated in Fig. 2.

dose is integrated at the target receptors to produce biologically significant damage. This approach fails to consider those agents that do have a concentration threshold below which toxicity does not occur. By so doing, the TWA concentration may represent an inappropriate measure of toxicologically significant target tissue dose. Conversely, selection of daily peak concentration as a metric assumes that it is only the peak concentration of an agent that induces a toxic response and thus ignores the possibility that there are a set of concentration values, greater than some dose-response threshold but less than the peak daily concentration, that are biologically active. Thus, the TWA and peak-value metrics represent maximally divergent alternative assumptions that together reflect the complete range of plausible toxic mechanisms, and either metric may be more appropriate, depending on a particular chemical's mechanism of toxicity (Urquhart *et al.*, 1984). Unfortunately, in the case of MC there is insufficient information at present to support selection of one approach over the other.

Following definition of the animal NOAELs ($D_{a,i}$) and selection of the plausible agents (A_j) and metrics (M_k), the PBPK model for rodents was used to derive corresponding effective doses in animals ($E_{a,ijk}$). We then made the assumption that each of the $E_{a,ijk}$ is equivalent to a corresponding effective dose in humans, $E_{h,ijk}$, and used the PBPK model for a reference 70-kg adult to calculate a corresponding applied dose to humans, $D_{h,ijk}$, which in turn is defined here as a function of the concentration of MC in drinking water that is predicted to result in the corresponding effective dose $E_{h,ijk}$ using the PBPK model and the multiroute exposure model discussed below.

OAELs

DERIVATION OF ALTERNATIVE TOXICOLOGICALLY EFFECTIVE DOSES

We followed the PBPK paradigm portrayed in Figs. 2 and 3 in order to calculate the toxicologically effective dose of MC in experimental animals, as described above. In the following paragraphs we discuss in detail the selection of agents and metrics associated with NOAELs from each of the experimental data sets selected above for inclusion in our dose–response assessment.

McNutt et al. (1975). These data characterize a NOAEL of 250 ppmv in mice exposed continuously to MC by inhalation for 14 weeks. We assumed that liver is the target tissue and that the process of MC metabolism (i.e., the daily amount of MC metabolized per kilogram of liver tissue) was the agent responsible for the hepatic toxicity observed by McNutt *et al.* at an exposure concentration of MC greater than the NOAEL identified by this study. This assumption was based on the data of Carlson (1973), who reported that pretreatment of rats with phenobarbital (50 mg/kg ip 4 days) potentiated the hepatotoxicity of inhaled MC (11,600 ppmv, 2 hr). Because McNutt *et al.* used a continuous exposure protocol over a 3-month period, the selected effective dose metric, the amount of MC metabolized per day per kilogram liver, or $\dot{A}_m$, can be assumed to have reached a virtual steady-state value for all but the first several days of the study. The rate of MC metabolism, $\dot{A}_m$, is given by Eq. (5) and the steady-state value of venous liver blood concentration (B_l) necessary to calculate $\dot{A}_m$ at steady state is given by Eqs. (10a)–(10d). McNutt *et al.* (1975) did not specify the age or body weight of experimental animals, and to apply the PBPK model

extrap-

to their data we made the assumption that the CF-1 mice used in this study were similar to the reference animals (young mice) of Reitz *et al.* (1987). Accordingly, we used the parameter values for mice given by Reitz *et al.* (1987) listed in Table 5. Using these values, B_1 = 14.5 mg/liter and $\dot{A}_m$ = 0.0154 mg/hr at steady state. To calculate the effective daily dose of MC to the mice exposed continuously to MC at 250 ppmv, we divided $\dot{A}_m$ (mg/hr) by the target tissue (liver) weight (kg), and multiplied by 24 hr/day to obtain a value of E_a = 319 mg/kg-day, in terms of liver weight.

Rosengren et al. (1985). To evaluate the toxicity data of Rosengren *et al.*, we chose the set of extrapolation options that correspond to $\{A_1, M_1\}$ and $\{A_1, M_3\}$ in Fig. 3. In this study, gerbils were exposed continuously to the NOAEL concentration of 70 ppmv MC for 3 months. Because of a lack of data on potential CNS effects of the metabolites TCA and TCEL, we made the assumption that the observed increase in GFA protein in the brain of gerbils exposed to more than 70 ppmv MC was due to the action of MC itself. Arterial blood was selected as a surrogate for the target tissue because MC has been detected in human and animal brain tissue after inhalation exposure and appears to diffuse readily across the blood-brain barrier. In the absence of specific information regarding the mechanism of MC-induced neurotoxicity, we were unable to rule out either the TWA concentration of MC in arterial blood or the corresponding daily peak concentration at dynamic equilibrium as an inappropriate metric. Therefore, we consider both approaches. Under the exposure conditions of this study (continuous, for 3 months), the tissue and blood concentrations of MC clearly reached virtual steady state for the majority of the exposure period. The steady-state, effective surrogate dose B_a (mg/liter) was calculated from Eq. (8) using the PBPK parameter values for gerbils listed in Table 5, which were presumed to be identical to the corresponding values listed for mice in that table. Application of the PBPK model to the exposure scenario of Rosengren *et al.* gives a value of B_a = 4.05 mg/liter, which is our estimate of the effective dose E_a received by the gerbils in this study.

NTP (1987b). To apply the PBPK model for rats to the NTP (1987b) data, it was necessary to make estimates of the average maternal body weight as well as approximations concerning the pattern of dosing. An average body weight of 0.344 kg for pregnant rats exposed to MC was calculated by averaging the body weights measured at Days 0, 6, 14, and 20 of gestation, and at parturition. PBPK parameter values for these rats were presumed to be the same as those used by Reitz *et al.* (1987) listed in Table 5, except that W was set to 0.344 kg and Q_a, Q_b, and V_{max} were all multiplied by $(W/0.215)^{0.7}$ to adjust for the difference in weight between the rats used by Reitz *et al.* and those used by NTP (1987b).

The average concentration of MC in drinking water during premating and gestation was 8.5 ppm for the 10 ppm treatment group. The NTP (1987b) calculated the average intake of MC by pregnant rats to be 1.16 mg/kg-day, implying a total daily intake of 0.40 mg/day. As noted by NTP (1987b), Armstrong (1980) reviewed the pattern of drinking water consumption by rats and found that when animals were maintained on a cycle of 12 hr light followed by 12 hr dark, 86% of the water was consumed during the dark period and 14% during the light period. Water consumption was bimodal during the dark period, but changed to a pattern of frequent ingestion of small amounts during the period of light. Thus, for the present analysis, we assumed that intermittent consumption of water occurred during the night, contrib-

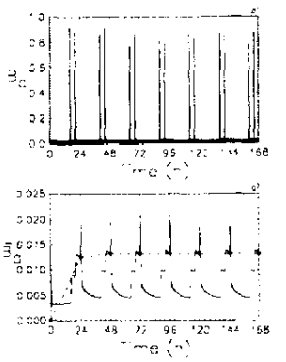

FIG. 4. (a) Arterial blood concentration, B_a (mg/liter), of methyl chloroform in rats used in the NTP (1987b) reproductive toxicity study, as predicted by a PBPK model (see text for details). (b) Periodic (24-hr cycle) time-weighted average (TWA) value of B_a, here denoted $\bar{B}_a$, in mg/liter; points connected by dotted line represent the predicted rolling 24-hr TWA average value of B_a.

uting 14% of the total dose (divided evenly into hourly 3-min ingestive pulses assumed to be injected at a constant rate directly into the liver compartment), and that the remaining 72% of the dose was divided into two similar 3-min periods of ingestion evenly spaced within the dark cycle.

In order to calculate an effective dose from the NTP study, we again made the assumption that MC itself, and not a metabolite, was the ultimate cause of suspected fetal toxicity. The placenta is relatively ineffective at screening compounds with a molecular weight less than 600 (Klaassen *et al.*, 1986), and there is no evidence to suggest that MC would not enter fetal circulation via the blood of the mother. Our PBPK model does not include a fetal compartment, so maternal arterial blood concentration, B_a, was used as a surrogate for effective fetal dose, E_a.

Because available data do not clearly define the most appropriate metric for effective dose for this study, we used both TWA and peak daily value of B_a as alternative plausible metrics. Numerical simulations were carried out for a 7-day period, and virtual dynamic equilibrium was reached for peak daily value of B_a after approximately 3 days (Fig. 4). Values for daily peak B_a and 24-hr TWA B_a were taken from Day 7 of the PBPK simulation. Figure 4 presents the results of our PBPK simulation of B_a in rats exposed to MC under the conditions of the NTP (1987b) study. Part (a) of this figure shows the daily peak B_a, which is largely determined by the two large nocturnal doses of MC. Use of the daily peak B_a value as the metric for effective dose gives an effective dose value of $E_a = 0.926$ mg/liter using the input dosing scenario described. Alternatively, use of the 24-hr TWA concentration of MC in arterial blood, $\bar{B}_a$, as the metric results in the substantially lower value of $E_a = 0.0132$ mg/liter.

DERIVATION OF ACCEPTABLE CONCENTRATIONS FOR MC IN DRINKING WATER

We applied the five-compartment PBPK model to a multiroute human-exposure scenario (described below) to derive a set of alternative acceptable concentrations in

water (ACWs) for MC. Within the PBPK paradigm (Figs. 2 and 3) an ACW is defined as the concentration of MC in water that, in conjunction with an appropriate human-exposure scenario, yields an effective dose E_h equal to E_a/SF, where SF is an appropriate safety factor. For purposes of comparison, we also calculated corresponding ACW values obtained by using a traditional approach to interspecies-toxicity extrapolation (see Fig. 2). The fundamental distinction between the two approaches is that the traditional method assumes equivalence between a NOAEL or nontoxic applied dose (D_a) to animals and a corresponding ENAEL in humans (D_h), i.e., that $D_h = D_a$ or $D_h = D_a$/SF, whereas the PBPK approach uses a PBPK model and an experimentally observed NOAEL to derive a nontoxic effective dose to a target tissue in animals (E_a) that is presumed to be equivalent to the corresponding effective dose in humans (E_h). If a safety factor is applied in the context of the PBPK approach, it is applied to E_a to yield a conservative value of E_h; the safety factor is *not* applied to D_a as it is in the traditional approach. Finally, the PBPK approach reapplies a PBPK analysis using exposure and pharmacokinetic models for humans to estimate that level of human exposure or applied dose (D_h) that is required to produce a corresponding value of E_h, where the calculation of E_h may have involved the use of a safety factor.[2]

The standard input exposure scenario used in our dose–response extrapolations was based on the multiroute exposure model for VOCs described by McKone (1987). To simplify the analysis, however, the model used here is a single parameterization applied only to a modeled 70-kg adult, rather than a set of full lifetime-exposure scenarios based on alternative exposure-model parameters. Nevertheless, the conservatism inherent in the daily exposure scenario for adults that we used is very likely to offset any greater sensitivity that might be predicted for infants or children from the PBPK analysis with which our exposure simulations are integrated.

The exposure scenario used was based on the assumption that a 70-kg adult spends 24 hr/day indoors in a household using water as described by McKone (1987). Daily average concentrations of 0.015 and 0.64 ppmv for MC in household and bathroom air, respectively, arising from tap water containing 1 mg/liter MC are assumed, along with a corresponding maximum air concentration of 4.8 ppmv in the shower compartment after 40 min of continuous use. The modeled exposure day begins with 1.5 hr spent in the household (i.e., non-bathroom/non-shower) compartment, followed by 18 min in the bathroom, 12 min in the shower, 12 hr in the household, 18 min in the bathroom, and, finally, 9.7 hr in the household. The time in the shower is presumed to be the final 12 min of 40 min of continuous shower operation using a total of 300 liters of tap water. For exposure simulation purposes, MC concentration in shower air over time was broken into two 6-min intervals, the first and second of which were set equal to 85.3 and 100% of the predicted peak air concentration referred to above. The latter assumption yields a 12-min TWA concentration of MC in shower air equal to 92.6% of its peak value after 40 min of shower use, which is

[2] Generally, in either the PBPK or the traditional approach, a safety factor of 10 is applied to account for intraspecies variability and an additional factor of 10 is used when extrapolating from animal studies of less than chronic exposure duration (U.S. EPA, 1980, 1985a,b, 1986b, 1988a; Dourson and Stara, 1983). In applying the PBPK approach, an additional factor of 10 might be applied to account for uncertainty due to PBPK parameter-estimation error. In the traditional approach, a safety factor of 10 is also used to account for interspecies variability, whereas the PBPK approach directly addresses the issue of interspecies-toxicity extrapolation and so would not require an additional uncertainty factor in this regard.

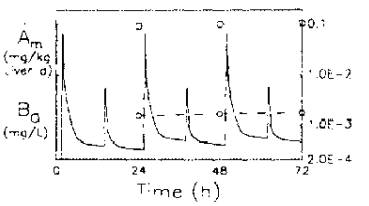

FIG. 5. Uptake and metabolism of methyl chloroform (MC) in humans predicted by a five-compartment PBPK model using as input 72 hr of a daily household exposure scenario involving respiratory, ingestion, and dermal exposure to MC present at 1 mg/liter in tap water. Solid curve = concentration (B_a) of MC in arterial blood; circular points = daily TWA values of B_a; square points = daily rate ($\dot{A}_m$) of MC metabolism.

predicted by the McKone (1987) household model using his "best" estimates for air-exchange rates. Presuming an alveolar ventilation rate of 378 liters/hr (from Table 5) and an MC concentration in water of 1 mg/liter, the respiratory exposure scenario described results in an applied dose (i.e., respired amount) of approximately 3.34 mg/day MC.

Dermal absorption of MC during showering was presumed to occur in accordance with the dermal uptake model for showering adults described by McKone (1987). Thus, dermal uptake during the modeled daily 12-min shower with water containing 1 mg/liter of MC was presumed to occur at a rate of (0.001 liter/cm^2-hr $\times$ 0.40 $\times$ 18,200 cm^2) = 7.28 mg/hr, resulting in a total dose of 1.46 mg to the skin compartment in our PBPK model over the 12-min showering period.

Finally, in our standard daily exposure model we assumed that 2 liters of tap water is ingested immediately before showering every day, and that the MC in the ingested water is completely absorbed (and transported directly to the liver—see discussion of the PBPK model) at a constant rate over a period of 6 min. Thus, with 1 mg/liter of MC in the ingested water, an ingestive infusion of 20 mg/hr to liver was assumed for the first 6 min of the shower-exposure period. Adding to this applied ingestive dose the applied respiratory and dermal doses referred to above results in a total daily applied dose of 6.8 mg implied by our reference multiroute exposure model.

The elements of the exposure scenario just described that contribute to conservatism in all "safe" drinking water concentrations calculated below are the presumptions of (1) continuous 24-hr residence in the home, (2) daily consumption of 2 liters of tap water, and (3) daily showering during the final 12 min of continuous 40-min shower use (i.e., when the shower-air concentration has built up to its highest predicted levels due to previous shower use). The presumption that daily ingestion of tap water occurs directly prior to showering adds a further degree of conservatism for those "safe" concentrations whose calculation is based on predicted daily peak concentration of MC in blood.

We applied the PBPK model to the exposure scenario just described using the parameter values for a reference 70-kg human from Table 5. The multiroute exposure simulations were run using different input tap-water concentrations to estimate the target-tissue dose in humans equal to E_a/SF, where E_a was derived by applying rodent PBPK models with input doses set equal to the NOAEL values based on the data of McNutt *et al.* (1975), Rosengren *et al.* (1985), and NTP (1987b) discussed earlier.

TABLE 7

PREDICTED NONTOXIC CONCENTRATIONS (IN mg/liter) FOR MC IN DRINKING WATER BASED ON A PBPK APPROACH[a]

Study	Toxic endpoint (effective dose metric)	Effective dose ($E_a = E_h$)	Predicted nontoxic concentration (mg/liter) using a safety factor of 1	100	1000
NTP (1987b)	Repro. (peak B_a)[b]	0.926 mg/liter	14	0.14	0.014
NTP (1987b)	Repro. (TWA B_a)[c]	0.0132 mg/liter	7.1	0.071	0.0071
Rosengren *et al.* (1985)	CNS (peak B_a)[b]	4.05 mg/liter	59	0.60	0.060
Rosengren *et al.* (1985)	CNS (TWA B_a)[c]	4.05 mg/liter	(2150)[d]	22	2.2
McNutt *et al.* (1975)	Liver (TWA mg/kg-day)[e]	319 mg/kg-day	(∞)[f]	36	3.0

[a] Each water concentration listed represents the concentration of MC in water (mg/liter) that, using a PBPK approach (see text for details), yields an effective dose equal to $E_h = E_a/SF$, where SF is an appropriate safety factor (e.g., 100 or 1000).

[b] Peak B_a = peak arterial blood concentration of MC.

[c] TWA B_a = 24-hr time-weighted average arterial blood concentration of MC.

[d] This concentration is impossible to achieve, given the solubility of MC in water of 1400 mg/liter.

[e] TWA mg/kg-day = 24-hr time-weighted average of MC metabolized per kilogram liver.

[f] Even if the limit on MC solubility in water were not considered, no concentration of MC in water could result in $E_h = 319$ mg/kg-day in this case, because the maximum daily amount metabolized in humans is expected (see Table 5) to be $V_{max}/1.82$ kg = 273 mg/kg-day, regardless of the applied dose level.

Figure 5 depicts the uptake and metabolism of MC predicted by the five-compartment PBPK model for 72 hr of the reference household exposure scenario described above with MC present in tap water at a concentration of 1 mg/liter. Although Fig. 5 shows the 24-hr TWA rate ($\dot{A}_m$) of MC metabolized and the 24-hr TWA concentration of MC in arterial blood (B_a) determined over a 3-day period, our simulations showed that approximately 20 simulated exposure days were required for these values to reach virtual dynamic equilibrium. As a consequence, our analysis and derivation of ACWs for MC were based on PBPK output for the 20th simulated exposure day.

The results of our analysis are summarized in Table 7, which presents a set of alternative ACWs for MC. Calculation of each ACW was done by iterative numerical optimization based on the exposure scenario and PBPK analysis described above. Note that the relationship among the values appearing in each row of ACWs in Table 7 is slightly nonlinear, due to the (nonlinear) Michaelis–Menten kinetics governing metabolism presumed by the PBPK model applied. This nonlinearity works in opposite directions depending on whether the presumed effective dose metric involves MC concentration in blood or the rate of MC metabolism, since metabolized MC is subtracted from the residual of parent compound that equilibrates between blood and tissues. Note also that the ENAEL for humans (i.e., D_h when SF = 1) corresponding to the hepatotoxic endpoint considered is infinite, due to the limit on potential metabolism implied by the value of V_{max} estimated for humans.

TABLE 8

PREDICTED NONTOXIC CONCENTRATIONS (IN mg/liter) FOR MC IN DRINKING WATER BASED ON A TRADITIONAL APPROACH[a]

Study	Toxic endpoint	Applied dose D_a (mg/kg-day)	Predicted nontoxic concentration (mg/liter) using a safety factor of		
			1	100	1000
NTP (1987b)	Fetal mortality	1.162[b]	41	0.41	0.041
Rosengren *et al.* (1985)	Increase GFA protein	322[c]	(11,000)[d]	110	11
McNutt *et al.* (1975)	Hepatic changes	1420[c]	(50,000)[d]	500	50

[a] Based on a traditional approach to interspecies toxicity extrapolation, each alternative concentration in water listed represents that concentration which, when multiplied by (2 liters/day)/70 kg, yields an applied dose of $D_h = D_a/SF$, where SF is an appropriate safety factor (e.g., 100 or 1000).

[b] Average applied dose to rats in the 10-ppm treatment group.

[c] Values calculated based on body weights and alveolar ventilation rates listed in Table 5.

[d] These concentrations are impossible to achieve, given the solubility of MC in water of 1400 mg/liter.

Without considering ACWs based on the NTP (1987b) toxicity data (whose biological significance should be interpreted with caution), the results of the PBPK approach summarized in Table 7 yield a 50- or 60-fold range in ACW values using a safety factor of 100 or 1000, respectively. Adding the ACWs based on reproductive toxicity increases the latter range by a factor of about 8.

For the purpose of comparison, a set of alternative concentrations for MC in drinking water based on a traditional approach to interspecies toxicity extrapolation is provided in Table 8. This method assumes that a 70-kg human adult consumes 2 liters of water in a 24-hr period and that exposure occurs only by ingestion. Those ACWs in Table 8 based on a safety factor of 100 or 1000 exhibit over a 1000-fold range, which is reduced to a 5-fold range if ACWs based on the reproductive toxicity data are not considered. To adjust these concentrations of MC to corresponding values that account for multiroute exposure, the concentrations in Table 8 would be divided by a factor of 6.8 mg/[(2 liters)·(1 mg/liter)] = 3.4, corresponding to the multiroute exposure model used here for dose-response extrapolation.

DISCUSSION

Those ACWs from Table 7 based on safety factors equal to 100 or 1000 are substantially lower (by factors ranging from approximately 3 to 180) than the corresponding ACWs based on the same toxicity data from Table 8. This discrepancy is due partly to the larger MC exposure predicted by the multiroute as opposed to the traditional exposure model, partly to the conservatism of the peak-concentration metric for effective dose, but also partly to the fact that the traditional approach fails to consider the degree to which applied doses of MC are lost through respiratory excretion, which in turn serves to decrease the effective doses involved. With regard to the latter factor,

the PBPK approach is more appropriate for interspecies dose-response extrapolation for VOCs, and for MC in particular, because it explicitly relies on comparing effective doses to a target tissue rather than total applied doses which may not be completely retained in the body.

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